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Strawberry-fortified Yogurt: Production, Sensory, Antioxidant Activity Test, and Model for Practicum

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ABSTRACT

This study aims to produce as well as analyze the sensory and antioxidant activity of fortified strawberry yogurt with cryoconcentration and pasteurization preparation methods. The results for the sensory test of strawberry fortified yogurt showed that the pasteurized yogurt is the best for taste and aroma because the use of heat when pasteurizing releases the aroma from strawberries. Reducing the water content using this method allows the strawberry flavor more concentrated. The cryoconcentration method allows the texture and color to be better than other methods. This is influenced by the use of low temperatures in the cryoconcentration method to maintain the anthocyanin content in strawberries which acts as a red pigment. The greater concentration of fortificants added results in higher antioxidant activity gained. Based on the IC100 value, the fortification of strawberries with the cryoconcentration method was significantly different from the pasteurization method in increasing the antioxidant activity of strawberry-fortified yogurt. The benefit of this research is to process milk into yogurt. Thus, it can be consumed by consumers with lactose intolerant sufferers. The addition of strawberries increases the antioxidants to prevent degenerative diseases. In addition, due to its simply design and production, this study is possibly used as a model in the practicum class.

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1. INTRODUCTION

Yogurt is a functional drink, fermented by milk using lactic acid bacteria, consisting of Streptococcus thermophilus and Lactobacillus bulgaricus. These probiotic bacteria maintain the balance of intestinal microbes to maintain health, reduce cholesterol levels and prevent diarrhea (Hendarto *et al.*, 2019). The addition of fortification ingredients can increase the nutritional content and function of yogurt (Raikos *et al.*, 2019). Strawberries (Fragaria x ananassa) are a fruit with complete nutritional content, as well as phenolic compounds, especially anthocyanins as a source of antioxidants (Ahouagi *et al.*, 2021; Yang *et al.*, 2021) in addition, strawberries have unique sensory properties because have the scent of bright red color, sweetness, juicy texture (Rahman *et al.*, 2021), and they are rich in vitamins and essential elements (Mousavi *et al.*, 2021). In yogurt sensory properties have a significant effect on consumer acceptance (Hossain *et al.*, 2020). A sensory test is a sensory assessment using the five human senses to observe the texture, color, aroma, and taste of a product, using measurements that are quantitative or qualitative (Faisal *et al.*, 2019; Nazir *et al.*, 2018). Sensory testing is carried out to determine the level of preference for a product seen from several sensory parameters including taste, aroma, texture, and color with a quality scale score of 1-5, according to the level of preference (hedonic) (Beegum *et al.*, 2019). Research related to the production of strawberry yogurt using the cryoconcentration method has been carried out, and the results show that the addition of strawberries to yogurt increases antioxidant activity and changes in physicochemical properties (Jaster *et al.*, 2018). Meanwhile, research on strawberry fortification using the pasteurization preparation method has also been carried out. And, the results showed changes in physicochemical properties and antioxidant activity when compared to yogurt without fortification (Sengül *et al.*, 2014). Fortification of yogurt using other natural fortifications have been carried out by several researchers such as fortified yogurt with grape seed extract, with the results showing an increase in antioxidants in fortified yogurt (Chouchouli *et al.*, 2013). Research conducted on the pumpkin and strawberry-fortified yogurt showed an increase in antioxidant and mineral content in yogurt (Atallah, 2015), while another study, regarding the addition of raspberries, the results showed an increase in the antioxidant activity of yogurt without reducing its physicochemical properties (Gunenc *et al.*, 2015).

This study aims to produce and analyze the sensory and antioxidant activity of strawberry-fortified yogurt with cryoconcentration and pasteurization preparation methods. In this study, the fortificants used were strawberries which were prepared using two different methods, the first method was cryoconcentration (block freeze concentration), namely the technique of concentrating fortifications at low temperatures to avoid degradation of phenolic compounds in the fruit (Jaster *et al.*, 2018), the second method of pasteurization, namely concentration and prolonging shelf life. store the fortification at high temperatures. Both of these preparation methods will affect the antioxidant content and physicochemical properties of yogurt (Gonçalves *et al.*, 2018), and affect sensory properties such as color, taste, and aroma (Mashiane *et al.*, 2021). The novelty of this research is the sensory test of strawberry yogurt with two kinds of preparation methods. Thus, the effect of the two methods on the sensory test of strawberry yogurt will be known.

This study is also possible to be used in the practicum class relating to the biotechnology and biochemistry classes due to its simple experiment, design, and production. This relates to the fact that the class must be

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supported by experiments and practicums (Ana, 2020; Nandiyanto *et al.*, 2018; Nandiyanto *et al.*, 2020; Hidayat *et al.*, 2020).

2. MATERIALS AND METHODS

The materials used in this study were pasteurized cow's milk (UHT), lactic acid bacteria starter consisting of Lactobacillus bulgaricus and Streptococcus thermophilus bacteria, strawberries, and water.The research phase begins with the production of yogurt. Milk was pasteurized at 85°C for 30 minutes, cooled to a temperature of 42°C, and added with a starter consisting of Streptococcus thermophillus and Lactobacillus Bulgaricus (5%). The mixture was incubated at 42°C for 8 hours and refrigerated until it is ready to use. The strawberry pulp preparation was followed by two methods, namely the cryoconcentration and the pasteurization method. The cryoconcentration method is the concentration of strawberry pulp by freezing strawberry juice at a temperature of 16 ± 2°C for 24 hours, and thawing it to 50% of the volume at room temperature.

The freezing and thawing processes were carried out 3 times to obtain a concentrated strawberry pulp. The pasteurization method was carried out by heating 250 g of strawberries with 50 mL of water added at a temperature of 65 ± 2°C until the strawberries were crushed. The process was then following the filtration. The strawberry filtrate was then pasteurized at a temperature of 90 ± 1°C for 5 minutes. Strawberry yogurt production consisted of several concentrations of strawberry pulp (see **Table 1**).

Analysis of the results was carried out by sensory tests, which included taste, aroma, texture, and color, which were tested on 20 untrained researchers. The panelists gave a rating according to a hedonic scale (1=dislike very much, 2=dislike, 3=moderately, 4=like, 5=very much like). The data obtained were analyzed by statistical tests using the ANOVA (analysis of variance) test, followed by Duncan's test with the help of the IBM SPSS application. Detailed information for the t-test analysis using SPSS is explained in elsewhere (Afifah *et al*., 2022).

For strawberry fortified yogurt antioxidant activity test, a total of 0.5 mL of the sample was put into a 10-mL volumetric flask, standardized with ethanol to 10 mL, taken 4 mL, and transferred to a dark vial. A total of 0.5 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder was weighed, dissolved with 25 mL of ethanol in a 25-mL volumetric flask, to obtain a standard solution of 50 ppm of DPPH. Next, the standard sample in a dark vial was added to 2 mL of 50-ppm DPPH solution. The bottles were tightly closed and incubated for 30 minutes at room temperature. The incubation mixture was put in a cuvette and tested for absorbance at a maximum wavelength of 517 nm.

Table 1. Code, composition, and method of preparation of strawberry yogurt.

No.	Code	Composition Stroberi: Yogurt g/g	Strawberry Preparation Method	Yield
1.	YK	0:200	-	Viscous, White
2.	K15	30:170	cryoconcentration	Viscous, reddish
3.	K30	60:140	cryoconcentration	Viscous liquid, red
4.	P15	30:170	Pasteurization	Viscous liquid, reddish
5.	P30	60:140	Pasteurization	Viscous liquid, red

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3. RESULTS AND DISCUSSION

3.1. Production of Yogurt

Pasteurization was carried out at a temperature of 85ºC for 30 minutes, aiming to kill pathogenic bacteria. Thus, the culture could grow optimally. During the pasteurization process, stirring was done to avoid coagulation of the milk, which causes the mixture to become homogeneous. Pasteurized milk was cooled to 42ºC and inoculated using Lactobacillus bulgaricus and Streptococcus thermophilus in a ratio of 1: 1 to obtain yogurt (see **Figure 1**). Lactic acid bacteria were added as much as 5% of the milk volume, and incubated at 42 C for 8 hours (Kennas *et al.*, 2020).

The resulting yogurt as shown in **Figure 1** is a white and slightly thick liquid with a characteristic sour smell. This is following the yogurt criteria in the previous study that the appearance of yogurt after fermentation is in the form of a thick-solid liquid and has a sour smell (Hendarto *et al.*, 2019).

Strawberry preparation using the cryoconcentration method started by making frozen strawberry juice (**Figure 2a**). After that, it was filtered to obtain a concentrated strawberry solution (**Figure 2b**). In the cryoconcentration method, there is a dehydration process, which allows the maintenance of the maximum sensory and nutritional quality of liquid food products, so this technique is suitable for separating chemical compounds that are susceptible to temperature. In the cryoconcentration method, two fractions were obtained. The first fraction was the concentrated fraction (solution), and the second was the ice fraction with low dry matter content. The efficiency of this process depended on the amount of dry matter in the ice (Jaster *et al.*, 2018).

Figure 1. Photograph image of yogurt produced.

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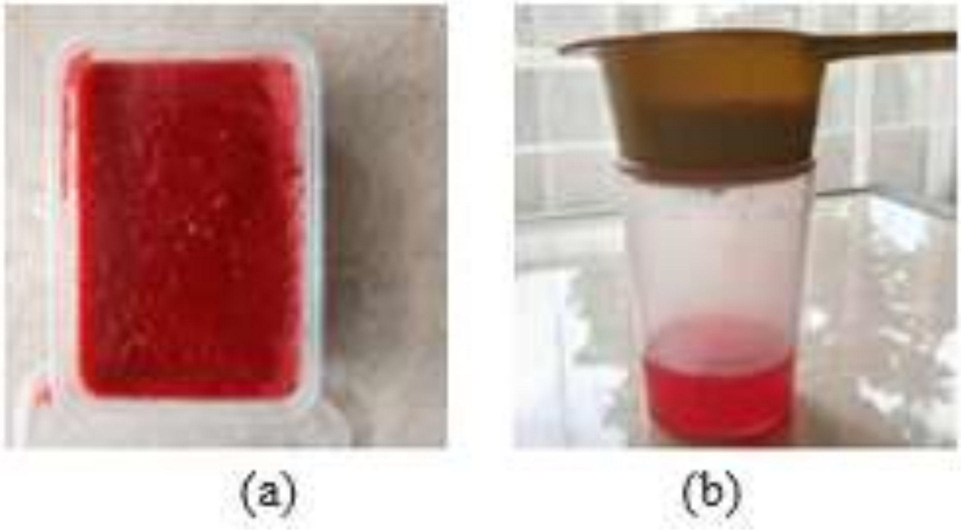

Figure 2. Photograph images of strawberry juice frozen for 24 hours (a) and concentrated strawberry solution (b).

The pasteurization method was started with the heat treatment to the strawberries. The heated strawberry was crushed and filtered to obtain strawberry juice and filter pulp (see **Figure 3**). Pasteurization involves the application of heat (less than 100°C) to food for a certain time. It can extend the shelf life of the product by eliminating or reducing microorganisms. It can also inactivate destructive enzymes. However, the intensity of the heat treatment can cause unwanted changes in the product, including appearance, color, flavor, taste, aroma, and thermal degradation of heat-sensitive compounds such as vitamins and another bioactive compound (Beegum *et al*., 2019). For some food products, the use of heat can increase the sensory attributes of the product. One of which is legumes (Bagheri *et al*., 2019). The results showed that the color of pasteurized strawberries was reduced, but the aroma of strawberries in this study increasingly came out after being pasteurized (Beegum *et al*., 2019).

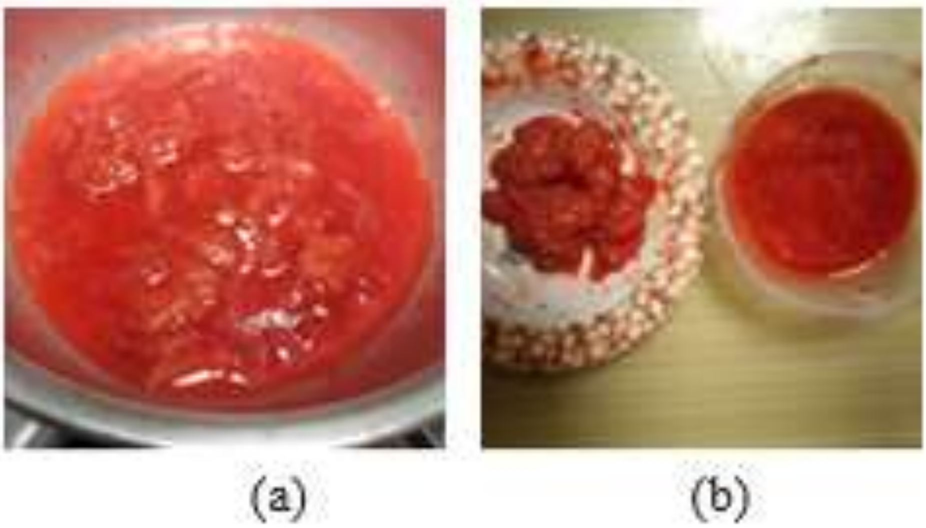

Figure 3. Photograph images of strawberries after pasteurization (a), strawberry pulp and juice (b).

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Strawberry yogurt products were made in four variants, and one yogurt without YK strawberries (control yogurt), as shown in **Table 1**. The addition of strawberry yogurt is thought to increase the antioxidant content. It is supported by the results of 15%of strawberry (K15) with cryoconcentration method can increase the antioxidant of 8.86±0.02 mgGAE/mL. For additional strawberry yogurt with 30% of strawberry with cryoconcentration method (K30) can increased antioxidant of 9.19±0.05 mgGAE/mL. Yogurt without strawberries (YK) has an antioxidant content of 0.00±0.00c mgGAE/mL (Jaster *et al*., 2018). Strawberry yogurt with the pasteurization method was proven to increase antioxidants according to the increasing concentration of strawberries added (Şengül *et al*., 2014). It can be concluded that the addition of strawberries using cryoconcentration and pasteurization methods can improve the function of yogurt in counteracting free radicals.

The production of strawberry yogurt made with two types of strawberry preparation methods was intended to determine the effect of the preparation method on the level of preference for strawberry yogurt products. The results of the analysis of the sensory attribute assessed including taste, aroma, texture, and color are shown in **Figure 4**.

Control yogurt (YK) is yogurt without the addition of any ingredients including sweeteners or other flavors. Thus, the sensory characteristics of this yogurt depended on the ingredients used, such as the type of milk and lactic acid content (Rohman & Maharani, 2020). In this study, the control yogurt produced was white, this result is following previous study that plain yogurt tends to be pale white (Rohman & Maharani, 2020). Strawberry-fortified yogurt has a reddish to red yogurt color. The intensity of the heat increases according to the concentration of added strawberries. The anthocyanin content in strawberries increases with the addition of strawberry fruit concentration as this compound acts as a pigment red color (Jaster *et al*., 2018). Based on **Figure 4**, the sensory test results for the color parameter showed that the value of strawberry fortified yogurt was higher than that of the control yogurt with the hedonic scale value for the color of the strawberry fortified yogurt is 3.50-4.45. The value of control yogurt (YK) was 3.00, meaning that the panelists' average prefers the color of fortified yogurt compared to control yogurt.

Control yogurt (YK) has a fairly sour taste, and aroma. The taste is caused by the presence of lactic acid fermented from starter lactic acid bacteria (Arifin *et al*., 2020; Rattu & Murali Krishna, 2020). Based on **Figure 4**, the panelists prefer the taste of strawberry-fortified yogurt compared to control yogurt. It can be observed from the average value of taste for control yogurt which is 2.35 lower than that for strawberry-fortified yogurt with cryoconcentration preparation and pasteurization methods with an average value from 3.00 to 4.00. Likewise, for aroma, from the results of the sensory test observations in **Figure 4**, the panelists prefer the aroma of the strawberry-fortified yogurt compared to the control yogurt. The average value of strawberry fortified yogurt aroma (K15, P15, K30, P30) was higher with a range of 3.50-4.30, compared to the control yogurt (YK)'s aroma with an average value of 3.20. The sour aroma of fortified yogurt is obtained from the content of vitamin C or citric acid, which is a distinctive and fresh citrus aroma. Thus, strawberry-fortified yogurt has a fresher and more distinctive aroma than control yogurt (Šic Žlabur *et al*., 2020).

The texture of control yogurt according to Indonesian standard (SNI 2981:2009) is a thick-solid liquid, this is following the results of control yogurt production, as shown in **Figure 4**. The texture of fortified yogurt is preferred by panelists compared to control yogurt because fortified yogurt has a texture that tends to be thicker. This is influenced by the addition of strawberries containing

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pectin which helps the gel formation process in yogurt (Drobek *et al*., 2020). Based on **Figure 4**, the addition of strawberries to yogurt improves sensory perception. This is supported by previous research stated that yogurt with the addition of strawberries has a sensory value above 3.6 for the attribute of color, appearance, aroma, and taste. This has a meaning that the average panelist liked strawberry-fortified yogurt (Arifin *et al*., 2020).

The sensory test data in **Table 2** has been statistically analyzed using SPSS software version 26 through the ANOVA test (analysis of variance) followed by the Ducan test to determine the significance of the data obtained. The results of statistical analysis are indicated by the measurement results, the same superscript letters in one column mean that there is no significant difference.

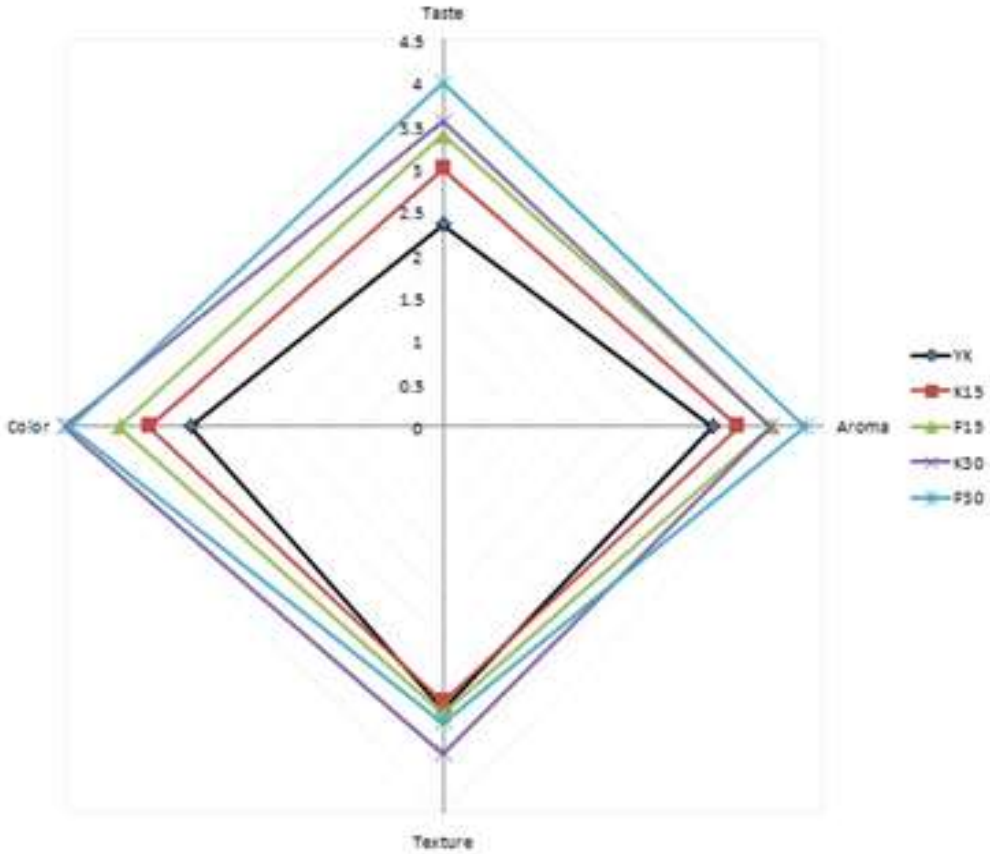

Figure 4. Sensory analysis spider diagram.

Table 2. Sensory test data for strawberry fortified yogurt.

Sample	Strawberry Porridge Concentration (%w/w)	Measurement results			
		Taste	Aroma	Texture	Color
YK	0%	3.00±0.65	3.20±0.70	3.30±0.86	3.00±1.12
K15	15%	3.00±0.65	3.50±0.95	3.20±0.83	3.50±0.82
P15	15%	3.40±0.94	3.90±0.97	3.35±1.09	3.85±0.74
K30	30%	3.55±0.68	3.90±0.64	3.80±0.83	4.50±0.51
P30	30%	4.00±0.92	4.30±0.47	3.45±0.99	4.45±0.60

Note: there is no statistically significant difference (p>0.05).

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3.2. Taste

Taste is the sensation felt by the taste buds when a substance enters the mouth (Beauchamp, 2019). The taste of fortified yogurt was preferred by the panelists, supported by the results of the Ducan test analysis which showed that there was a significant difference between the average value of the taste of strawberry fortified yogurt and the control yogurt. As shown in **Table 2** based on the preparation method, as long as the sample was added by strawberry, there is no significant difference in taste measurement results. The highest value was obtained for sample P30 (30% of strawberry with pasteurization preparation) reaching a value of 4.00. This type of yogurt tends to have a sourer taste than other samples. This is influenced by the sweet taste of strawberries, which is a combination of fructose, sucrose, and galactose (Lee *et al*., 2018). The activity of lactic acid bacteria converts fructose, sucrose, and galactose into lactic acid when fermentation takes place, making the sour taste of strawberry-fortified yogurt to be increased (Bintsis, 2018).

3.3. Aroma

In a food or beverage product, the aroma becomes an important parameter because it will affect the attractiveness of consumers before buying or consuming a product. Aroma is the smell caused by chemical stimuli that are smelled by the olfactory nerves in the nasal cavity, the aroma in yogurt is produced by the fermentation of lactic acid bacteria causing aroma and acid (Yang *et al*., 2021). Based on **Table 2** for the aroma parameter, the panelists stated that they liked the hedonic value of 4.30 for the aroma of the 30% of strawberry-fortified yogurt sample using the pasteurization preparation method (P30) which had a distinctive sour and fresh aroma. The sour aroma in fortified yogurt is obtained from the breakdown of glucose by lactic acid bacteria (Hoheneder, 2021). Lactic acid is produced in the amount of sugar available. In addition, strawberries have a distinctive aroma from the content of vitamin C or citric acid, namely a distinctive and fresh citrus aroma. Thus, strawberry fortified yogurt has a fresher and distinctive aroma than control yogurt (Arifin *et al*., 2020).

3.4. Texture

Yogurt texture determines the quality of the resulting yogurt. Generally, good yogurt has a soft texture like porridge, not too runny and not too thick (Faisal *et al*., 2019). The texture of the yogurt produced in this study was soft with various levels of viscosity, the control yogurt tended to be waterier than the strawberry-fortified yogurt. Based on **Table 2**, the yogurt texture that the panelists preferred was the K30 sample or the 30% strawberry fortified yogurt with the cryoconcentration preparation method which tended to be thicker in texture than the others. The texture of fortified yogurt is influenced by the pectin content in strawberries which helps the gel formation process in yogurt. In addition, the content of vitamin C, citric acid, and ascorbic acid increase the acidity of fortified yogurt. It helps protein coagulation in milk. Therefore, the addition of strawberry pulp causes yogurt texture becomes thicker (Rohman & Maharani, 2020; Ergün *et al*., 2021). The Duncan test results for texture attribute showed that control and strawberry-fortified yogurts did not have a significant difference in the panelists' preference level.

3.5. Color

Color is an important sensory parameter besides taste, aroma, and texture. Color is one of the factors that influence consumer acceptance of food products [8]. Yogurt with the addition of strawberry pulp has a pink color that comes from the anthocyanin content in strawberries. According to literature (Dzhanfezova, 2020), anthocyanins are water-soluble pigments that are naturally found in various types of plants and fruits.

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This pigment will give the strawberry its distinctive red color. The results of Duncan's analysis, control and strawberry fortified yogurts have a significant difference in the level of panelists' preference for color attribute. Based on **Table 2**, the color of the 30% of strawberry in the fortified yogurt with the cryoconcentration preparation method (K30) was preferred by the panelists. The color of the yogurt had a lighter red color than the other samples. The higher concentration of strawberry pulp added resulted in the lighter color of the yogurt (Jaster *et al*., 2018).

3.6. Antioxidant activity test results

The antioxidant activity test aims to determine the ability of antioxidant compounds in strawberry-fortified yogurt in counteracting free radical compounds. The test used the DPPH radical trapping method. This compound reacts with antioxidants to form a non-radical DPPH compound, which is characterized by a change in the color of the solution from purple to yellow. This occurs due to a decrease in the wavelength of the purple color to yellow (Liang & Kitts, 2014). The results of measurements of absorbance and antioxidant activity of strawberry-fortified yogurt are presented in **Table 3**.

Table 3 shows that the lower concentration of strawberries added to yogurt resulted in the higher absorbance value. This absorbance value is inversely proportional to the percentage of antioxidant activity produced. Thus, to get the best quality of antioxidants, more strawberry fortifications are needed. The measurement results of the IC100 value indicated that the antioxidant activity of strawberry-fortified yogurt using the pasteurization method is stronger in scavenging free radicals than in the cryoconcentration method. The smaller IC100 value resulted in the stronger antioxidant activity (Inggrid & Santoso, 2015). The IC100 measurement aims to determine the antioxidant activity of strawberry-fortified yogurt, which can inhibit 100% of free radicals (Suhery *et al*., 2016). The IC100 measurement used the regression equation from the results, shown in **Figures 5** and **6**.

The increase in antioxidant activity was influenced by the increasing levels of antioxidant compounds along with the high concentration of strawberry fortification added to yogurt. Anthocyanin is a compound that functions as an antioxidant. The mechanism of inhibition of DPPH free radical activity is by anthocyanins. Hydrogen atoms of anthocyanin compounds from some of their hydroxyl groups are donated to DPPH free radicals to form a more stable DPPH free radical compound (DPPH-H) (Handayani *et al.*, 2014). The more anthocyanin compounds, the more hydrogen atoms can be donated to inhibit the activity of DPPH free radicals.

Table 3. Calculation of antioxidant activity.

Preparation Method	Strawberry Fortification Concentration	Sample	Absorbance	Antioxidant Activity	Ic_{100}
-	0%	Yo	0.243	76.52%	-
Cryoconcentration	15%	K15	0.042	95.94%	41.24
	30%	K30	0.018	98.26%	
Pasteurization	15%	P15	0.044	95.75%	31.39
	30%	P30	0.029	97.78%	

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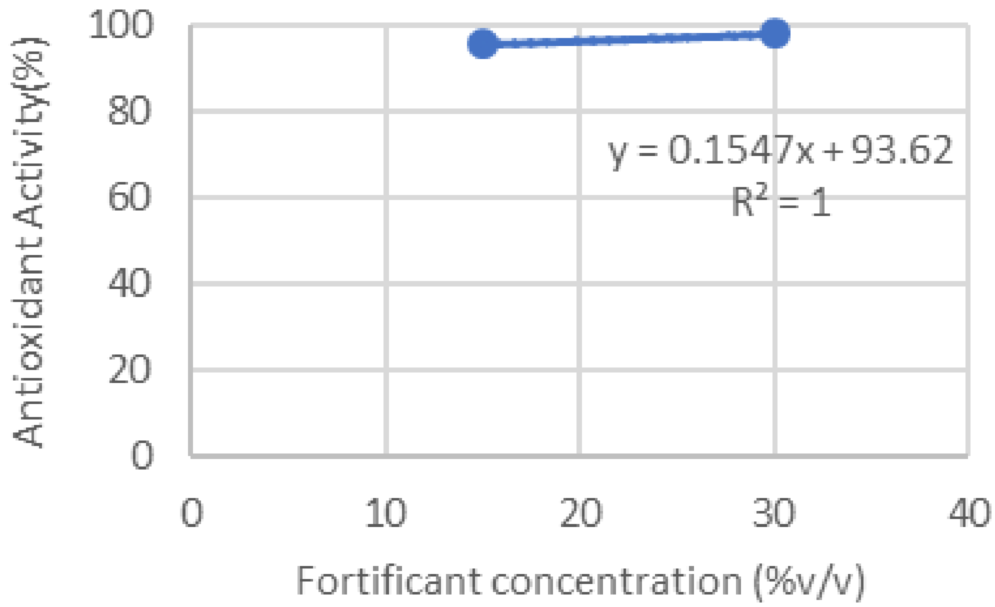

Figure 5. Correlation of Antioxidant activity with Fortificant Concentration using cryoconcentration method.

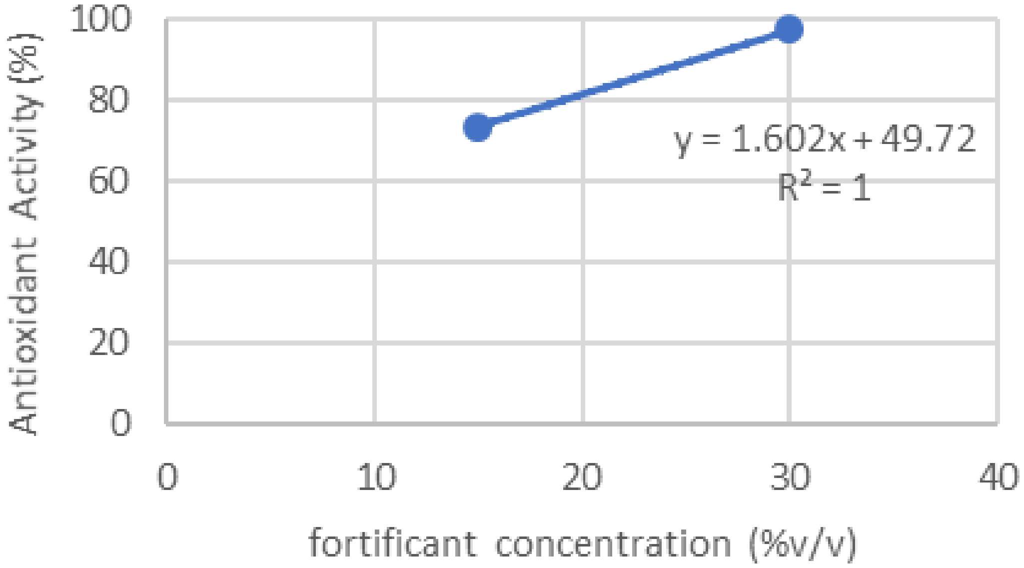

Figure 6. Correlation of Antioxidant activity with Fortificant Concentration using pasteurization method.

Significant testing using IBM SPSS version 26 software shows that the t-value is greater than the t-table ($p = 0.05$) in both fortified preparation methods. The t-value is 240.4 and the t-table is 12.71. Based on these results, the fortification of strawberries with the cryoconcentration method was significantly different from the pasteurization method in increasing the antioxidant activity of strawberry-fortified yogurt.

4. CONCLUSION

Four variants of strawberry yogurt have been made. From the sensory test for the attribute of taste, aroma, texture, and color, the panelists preferred fortified yogurt compared to control yogurt. Sensory test

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results of strawberry-fortified yogurt showed that the panelists preferred 30% of strawberry in the fortified yogurt with the pasteurization preparation method for the aroma and taste attribute. For the texture and color attribute, panelists preferred 30% of strawberry in the fortified yogurt with the cryoconcentration preparation method. The greater concentration of fortificants added resulted in the higher antioxidant activity. Based on the IC100 value, the fortification of strawberries with the cryoconcentration method was significantly different from the pasteurization method in increasing the antioxidant activity of strawberry-fortified yogurt.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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